recorded voltage ramps in the interval ±200 mV in the presence of 2 M internal sucrose. In these conditions, outward currents become voltage independent at voltages >150 mV, providing size estimates consistent with the size of the internal mouth derived from the crystallographic structure. The ion density profile obtained from molecular dynamics simulations using an applied voltage reveals a high density of K+ ions near P475D.
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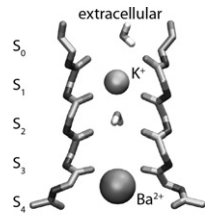
3064-Pos Board B169
Calculating Conductance and Size of the Entrance to the Inner Cavity of BK Channels with Side-Chain Replacement and a Two-Resistor Model
Yanyan Geng, Karl L. Magleby.
BK channels have the largest conductance (~250 pS) of all K+ selective channels. Previous studies suggest that residues E321/E324 in BK channels are located at the entrance to the inner cavity. We find that attachment of thiol reagents MPA and MTSET to E321C/E324C altered outward single-channel currents, suggesting that 321/324 face the ion conduction pathway. Therefore, substituting E321/E324 with different sized amino acids should change the size of the entrance to the inner cavity. We find that decreasing the size of the entrance decreases the conductance, whereas increasing the size of the entrance has little effect. Increasing [K+]i from 0.15 to 2.5 M negates differences in single-channel current associated with different side-chain volume. Plots of conductance vs. side-chain volume are approximated with a simple two-resistor model, where the ion conduction pathway is described by two resistors in series. R2 is a variable resistor, with resistance inversely proportional to the volume of the entrance to the inner cavity. R1 is a fixed resistor arising from the other parts of the conduction pathway including the selectivity filter. Fitting the data indicates that R1+R2 is ~5.4 gigaohm for glycine substitution, with an R1/R2 ratio of ~17, and effective radius and length of the entrance to the inner cavity of ~9.0 and 5.4 Å, respectively. (The volume of K+ and water are not taken into account.) The calculated size of the entrance to the inner cavity of BK channels is consistent with the crystal structure of large conductance bacterial MthK channels. These observations suggest that a large entrance to the inner cavity is required for the large conductance of BK channels, as decreasing the entrance size decreases the outward single-channel currents. Support: NIH-AR32805.

3065-Pos Board B170
Mechanism for Selectivity-Inactivation Coupling in KcsA Potassium Channels
Jason G. McCoy, Wayland Cheng, Ameer N. Thompson,
Crina M. Nimigean, Colin G. Nichols.
Potassium channels containing the GYG motif often diverge in their selectivity for monovalent cations, but the molecular basis is unknown. Using the prokaryotic potassium channel KcsA as our model, we have investigated the role of the interaction between glutamate 71 and aspartate 80, located behind the selectivity filter, in determining the selectivity of the channel as well as its influence on the conformation of the filter. In E71A KcsA channels, Na+ permeates at higher rates in both the presence and absence of K+, as seen with 86Rb+ and 22Na+ flux measurements. Single channel recordings indicate that Na+ ''punches through'' E71A KcsA channels at lower voltages than wild-type KcsA, and in the punchthrough regime the Na+-blocked current appears significantly larger. A crystal structure of E71A KcsA reveals that in contrast to what was seen for wild type KcsA, the selectivity filter does not collapse in the absence of K+, but instead assumes a ''flipped'' conformation. This flipped conformation is the same one observed in previous E71A KcsA structures in the presence of K+. The data reveal the importance of this E71-D80 interaction in both favoring inactivation and maintaining high K+ selectivity. We propose a molecular mechanism by which inactivation and K+ selectivity are linked, a mechanism that may also be at work in other channels containing the canonical GYG signature sequence.

3066-Pos Board B171
Structural Characterization of the Voltage Sensor Domain of the KvAP Channel Vectorially-Oriented within a Phospholipid Bilayer Membrane
Sanju Gupta, J. Liu, A. Tronin, J. Strzalka, J. Krepkiy, K. Swartz,
J. Kent Blasie.
Voltage-gated cation (Na^+, K^+) channels are responsible for the generation and propagation of action potentials in neurological signal transmission. Kv-channels are transmembrane proteins consisting of a homo-tetramer of 4 subunits that assemble about a 4-fold axis normal to the membrane plane to form the K^+ ion-selective pore. Each of the four subunits is comprised of six transmembrane helices, the S1-S4 helices forming the voltage-sensor domain (VSD) and the S5-S6 helices contributing to form the pore domain (PD). Despite several advances in the field, a complete understanding of the mechanism of electromechanical coupling interconverting the closed-to-open states is yet to be achieved. Positively charged arginine residues predominately in the S4 helix of the VSD are responsible for voltage sensing and the VSD's are arranged around the periphery of the PD in extensive contact with the lipid bilayer. This prompted us to focus initially on the structure of VSD itself within a phospholipid bilayer environment for the present study. A hydrated, phospholipid bilayer membrane environment has been reconstituted for the VSD of KvAP, vectorially oriented on the surface of inorganic multilayer substrates. This has been established by X-ray and neutron reflectivity (enhanced by interferometry), the latter employing a specifically deuterated phospholipid and water contrast variation, for the reconstituted membrane at both the solid-vapor and solid-liquid interfaces. This accomplishment now allows an investigation of the profile structure of the VSD within the lipid bilayer as a function of the applied transmembrane electric potential via x-ray reflectivity with millisecond time-resolution, employing high energy x-rays (> 20KeV) & pixel array detectors, and neutron reflectivity, employing selectively deuterium-labeled VSD proteins achieved via semi-synthesis. The same approach can be extended to the intact KvAP channel.

3067-Pos Board B172
KcsA Ion Affinity at an External Site Probed by Barium Block
Kene N. Piasta, Christopher Miller.
Block by Ba^{2+} is a distinctive property of K^+ channels since Ba^{2+}, a doubly charged analog for K^+, is electrostatically stabilized in the permeation pathway. Ba^{2+} block was used in BK channels as a tool to determine the equilibrium binding affinity for various ions at specific sites in the selectivity filter. In this work, we applied this approach to discrete block of single E71A KcsA channels, a non-inactivating mutant, in order to determine a thermodynamic measure of selectivity in a channel with abundant high-resolution structural information. We find at high concentrations of external K^+ the block time distribution is described by two distinct populations of Ba^{2+} block events. This argues there are at least two Ba^{2+} sites in the selectivity filter, fitting well with the published Ba^{2+} containing structure of KcsA where a Ba^{2+} ion resides approximately in S2 and S4. Utilizing a kinetic analysis of the blocking events as a function of external K^+, we determined the equilibrium dissociation constant of K^+ and other monovalent cations in an extracellular site, presumably S1, to arrive at a selectivity sequence for this particular site: Rb^+ (1 M) > K^+ (19 M) >> Na^+ (>1M). This represents an unusually high selectivity for K^+ over Na^+ with a $\Delta\Delta G^0$ of at least -7 kcal mol^{-1}. We are currently determining affinities for Li^+, NH_4^+, and Cs^+ at this site. The results fit well with other kinetic measurements of selectivity as well as with the many structures in various ionic conditions.

3068-Pos Board B173
QM/MM Modeling of Ba^{2+} Blockades in Potassium ion Channels
Christopher N. Rowley, Bogdan Lev, Sergei Noskov, Benoit Roux.
The robust selectivity of potassium channels for K^+ over Na^+ ions is a major component of the regulation of intracellular K^+ concentrations. The selectivity for K^+ was quantified through experiments measuring the Na^+ and K^+ dependence on Ba^{2+}-blockades,(1) indicating that K^+ has greater permeability by at least 150 fold. In thermodynamic terms, the relative binding free energy of Na^+ to the pore must be at least 3 kcal/mol less favorable than K^+. Na^+ vs K^+ free energy perturbation (FEP) simulations are consistent with this, although no simulations to date have modeled the actual Ba^{2+} blockade experiment. We have used MD simulations to calculate the relative binding energies of Na^+ and K^+ in the KcsA ion channel when the S4 site is occupied by Ba^{2+}. As Ba^{2+} is a strongly polarizing ion, we have used QM/MM FEP calculations using CHARMM interfaced to the deMon DFT code (2), as well as the polarizable Drude force field to correctly model the Ba^{2+}-filter interactions, with the aim of better interpreting the original selectivity experiments.



Schematic of ion binding in the selectivity filter.

1. J. Neyton, C. Miller, *J. Gen. Physiol.* 92: 549-567.
2. B. Lev et al., *J. Comp. Chem.*, 31: 1015-1023.

3069-Pos Board B174
Human *ETHER-A-Go-Go*-Related Gene (HERG) K^+ Channel Inhibition by the Antidepressant Paroxetine
Hee-Kyung Hong, **Su-Hyun Jo**.
Paroxetine is a selective serotonin reuptake inhibitor (SSRI) for psychiatric disorders that can induce QT prolongation, which may lead to *torsades de*